Online recording of ethane traces in human breath via infrared laser spectroscopy

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Von Basum, Golo, Hannes Dahnke, Daniel Halmer, Peter Hering, and Manfred Mürtz. Online recording of ethane traces in human breath via infrared laser spectroscopy. J Appl Physiol 95: 2583–2590, 2003. First published August 1, 2003; 10.1152/japplphysiol.00542.2003.—A method is described for rapidly measuring the ethane concentration in exhaled human breath. Ethane is considered a volatile marker for lipid peroxidation. The breath samples are analyzed in real time during single exhalations by means of infrared cavity leak-out spectroscopy. This is an ultrasensitive laser-based method for the analysis of trace gases on the sub-parts per billion level. We demonstrate that this technique is capable of online quantifying of ethane traces in exhaled human breath down to 500 parts per trillion with a time resolution of better than 800 ms. This study includes what we believe to be the first measured exipigograms for trace fractions of ethane. The expirograms were recorded after a controlled inhalation exposure to 1 part per million of ethane. The normalized slope of the alveolar plateau was determined, which shows a linear increase over the first 481, and 370 to 1,770 s. Our analyzer provides a promising noninvasive tool for online monitoring of the oxidative stress status.

among the various volatile hydrocarbons found in breath, the alkanes ethane (C2H6) and pentane (C5H12) have been extensively studied since they were identified as end products of the oxidative degradation (lipid peroxidation) of polyunsaturated fatty acids. The process of lipid peroxidation has gained interest as one of the important features of free radical-induced damage in biology and medicine (29). During peroxidation of omega-3 and omega-6 fatty acids, ethane and pentane, respectively, are formed and excreted via the lungs and thus can be detected in exhaled breath. Because lipid peroxidation is considered as the major, probably the only endogenous source of pentane and ethane, these volatile compounds may serve as specific markers for oxidative damage (8, 25).

The first report of breath ethane as a marker of in vivo lipid peroxidation was published by Riely et al. in 1974 (24). During the past decade, several studies provided evidence that ethane and pentane in exhaled air are useful markers of in vivo lipid peroxidation under certain clinical conditions (1–4, 8, 19, 20, 26, 27). Because pentane is metabolized in the liver, ethane is considered as the more reliable marker (10).

Despite the growing number of reports on breath ethane and pentane, the development of rapid and sensitive analysis techniques for measurements of these markers in exhaled breath still remains a challenge. Ethane and pentane fractional concentrations in expired air are in the parts per billion (ppb; 1:109) range, which is below the detection limit of most analytical methods.

The technique usually applied for quantifying these hydrocarbons in exhaled breath is gas chromatography (10). To detect ppb levels, the breath samples must be accumulated and preconcentrated up to 100-fold, e.g., by means of a sorbent trap, before subsequent desorption and analysis. This procedure plus the gas chromatography analysis is time consuming, and under several circumstances such measurements are prone to errors, as Kneepkens et al. (9) pointed out. The sorbent traps have to be carefully conditioned before usage, and the chromatographic column must be very carefully selected and prepared (7, 11, 27). The details of these off-line methods for collecting, identifying, and quantifying breath hydrocarbons have recently been reviewed (10).

To overcome the potential problems of off-line methods, like reproducibility of breath-sample collection, contamination during sample storage, and the inability to allow for instantaneous feedback, online methods are desirable. Online methods are characterized by real-time gas sampling and analysis; the resulting concentration together with other variables, like flow rate, are continuously captured and displayed without significant delay. This allows quasi-continuous monitoring of the gas sample; in the case of breath tests, for example, suboptimal exhalations can be immediately identified and discarded. Additionally, information about the ethane exhalation during different exhalation phases and the sloping alveolar plateau is directly accessible via fast online measurements, whereas offline methods integrate over a complete exhalation and...
require an extra effort to separate alveolar gas from dead-space gas, e.g., via CO₂-controlled sampling (28). However, up to now, there is no online technique available that allows real-time monitoring of ppb ethane traces in exhaled breath.

The aim of our present work was to develop and characterize a method for the precise quantitative online analysis of trace constituents in exhaled human breath on the ppb level. We have developed a laser-based spectroscopic technique with unique sensitivity and specificity, which provides a very high time resolution. Our group has previously reported precise monitoring of various trace gases by using cavity leak-out spectroscopy (CALOS) (15), a continuous-wave variant of cavity ring-down spectroscopy, which was developed in the late 1980s (17). Recently, our laboratory has reported on the offline analysis of ethane in human breath, where the breath collection was performed with sample bags (6).

In this paper, we characterize this technique regarding sensitivity and speed of ethane monitoring in exhaled human breath. In addition to the description of the analyzer, we show the first data of quantitative time-resolved breath ethane measurements during single exhalations. Data regarding the slope of the alveolar plateau are given. Additionally, we have studied the ethane washout process after exposition to increased levels of ethane.

MATERIALS AND METHODS

Experimental Design

Subjects. Three healthy, nonsmoking male volunteers (26–38 yr), who had no diagnosed chronic or acute disease of the respiratory tract, were selected. The ethane exhalation of all subjects under normal conditions was below detectable levels. Furthermore, the subjects had no medication for at least 3 days before the measurements. This study was conducted in accordance with the guidelines of the local institutional review board. Written, informed consent was obtained from all participants.

Breathing maneuvers. The experiments were performed at the Institute for Laser Medicine, University of Düsseldorf. During the whole measurement, subjects were calm and seated. Subjects performed defined breathing maneuvers, which were divided into two parts. In the first part of the investigation, individuals inhaled 1 parts per million (ppm) ethane in synthetic air (Messer Griesheim) for a duration of 5 min to enrich the subject with ethane (washin). This trace concentration is a factor of 30,000 below the explosive concentration of 3% in air. Until now, no maximum allowable concentration is defined for ethane (16). It should be noted that the subject was not equilibrated after 5 min of washin, but the goal of this study was to prove the suitability of our CALOS analyzer for very sensitive single-breath detection of ethane traces.

During the second maneuver, subjects inhaled and exhaled through a mouthpiece (modified Datex Ohmeda), which is used to direct portions of the breath into the CALOS analyzer and a commercial capnograph with combined spirometer (Datex Ohmeda, Capnomac Ultima). The capnograph is used to monitor the gas flow and the concentration of CO₂ and O₂. These data were digitally recorded via the analog output of the capnograph and an analog-to-digital converter in a personal computer. The subjects were asked to inhale and exhale four times per minute at a constant flow rate of 20 l/min, which means a tidal volume of 2.5 liters. This was achieved by means of a biofeedback loop.

In a second investigation, subjects went again through the first part of the breathing maneuver, but during the second part the consequences of exhalation beyond the functional residual volume were examined, meaning that the individu-

als exhaled to their residual volume.

Breath sampling. Breath sampling was performed by means of a modified mouthpiece, which is used to connect the inhaling and exhaling individuals with the analyzer. Two gas supplies at the mouthpiece were used to continuously extract portions of the breath into the CALOS analyzer and the capnograph.

A schematic of the complete gas setup is shown in Fig. 1. The gas flow through the CALOS analyzer is maintained by a rotary pump behind the absorption cell. The breath flow is determined by measuring the pressure difference before and behind a resistance inside the mouthpiece. For monitoring the CO₂ and O₂ concentrations, a part of the exhaled breath (200 ml/min) is extracted and fed to the capnograph. A second portion of 1,000 ml/min is extracted and directed into the CALOS analyzer. To avoid contamination with outgassing materials, all parts that are in contact with the gas flow are made of stainless steel, copper, or Teflon. The gas sample is dehumidified by means of a Naion tube (PermaPure, length of 2 m) and led through a cooling trap at a temperature slightly above liquid nitrogen temperature (−160 °K) to eliminate all interfering molecules (e.g., isoprene, pentane).

![Fig. 1. Schematic of the gas setup.](http://www.jap.org)
The amount of gas directed into the detection cell is controlled by means of a mass flow controller (1,000 ml/min). The pressure inside the cell was kept constant at 48.8 mbar independently of the flow. This was achieved by means of a control loop consisting of a pressure gauge and an electronic valve. The sample gas is injected in the middle and extracted on both sides of the absorption cell. The cell volume between the mirrors is 190 ml. With regard to flow, pressure, and volume, the system has a theoretical gas exchange time of 560 ms.

Technical Principles and Arrangements of the CALOS Spectrometer

In earlier studies, our laboratory showed the successful application of the CALOS analyzer (6). This work demonstrated highest sensitivity [detection limit for ethane: 100 parts per trillion (ppt) in 5 s] and specificity in environmental and medical tasks.

The detection method is based on the principle of absorption spectroscopy. For the analysis of ethane traces, the “fingerprint” spectrum of ethane in the midinfrared wavelength region is used. The concentration of ethane is determined from the light absorption observed at characteristic wavelengths.

Our CALOS analyzer employs a narrow line width continuous-wave laser in the midinfrared spectral region. It consists of a tunable CO overtone sideband laser and an absorption cell containing the gas sample of interest. Our absorption cell is a high-finesse ring-down cavity (length of 53 cm) excited by the laser radiation. Figure 2 shows a schematic diagram of the setup.

**Technical details.** The CO laser operates on ~300 rovibro-tional overtone transitions in the wavelength region between 2.6 and 4.0 μm with single-line output power in the order of 100 mW. The laser has been described in more detail by Murtz et al. (14). By mixing the laser light with microwave radiation in an electrooptic modulator, tuneable laser sidebands are generated, which cover a spectral range of 8–18 GHz above and below each laser line with a power of 50–150 μW. The sideband radiation excites the fundamental transverse mode of the ring-down cavity. The cavity mirrors have a reflectivity of \( R = 99.98\% \), which provides an effective optical absorption path length of >3 km. Frequency stabilization of the cavity resonance to the laser frequency is accomplished by means of a standard 1/f-lock-in technique. In this way, the laser power is periodically injected into the ring-down cell, twice per modulation period. Each time the transmitted light indicates optimum coincidence of laser frequency and cavity mode, a trigger pulse is provided to turn off the laser sideband radiation via the electrooptic modulator. The subsequent leak out of the light is monitored with an InSb photodetector and acquired by means of an analog-to-digital converter. The decay time of the leak-out signal is determined by fitting a single exponential to the data. By measuring the decay time of the empty cell (\( \tau_0 \)) and the decay time of the cell filled with the breath sample (\( \tau \)), the absorption coefficient and, therefore, the concentration can be directly determined by using the formula

\[
\alpha(\lambda) = \frac{1}{c} \left( \frac{1}{\tau(\lambda)} - \frac{1}{\tau_0} \right)
\]

where \( \alpha \) is the absorption coefficient, \( \lambda \) the wavelength, and \( c \) the speed of light.

**Data acquisition and processing.** For the online analysis of human breath, a subsecond time resolution is required. Therefore, fast data acquisition and processing are very important for the success of this analysis. The recorded exponential decay signals are passed to a state-of-the-art personal computer (CPU clock: 1.7 GHz) by means of a 12-bit analog-to-digital converter card (Gage, CompuScope 1250) with a sample rate of 25 MHz. The modulation of the laser frequency at 825 Hz leads to a signal generation rate of 1.65 kHz. This requires a fast exponential fitting routine that fits a single exponential signal with a sample length of 1,536 Pts within 500 μs. Because the internal Levenberg-Marquardt-fitting routine from the used programming language (LabView 5.0, National Instruments) needs 100 ms for this task, a new fitting routine based on the method of successive integration was developed in our group. This fitting routine only needs 150 μs for the same data set. Therefore, it is possible to obtain the decay time from each recorded exponential decay signal. The data are then smoothed by calculating the running average over \( N \) data points. \( N \) is chosen such that the time resolution is dominated by the gas exchange time and not worsened by the averaging routine. Typical values for \( N \) are on the order of 500.

**Statistical Methods and Analysis of the Data**

A representative example of an online recording is shown in Fig. 3. The uppermost curve displays the course of the...
breath ethane concentration detected with the CALOS analyzer. The curves below show the concentration of breath CO$_2$, O$_2$, and flow analyzed with the capnograph. During the washout period, subjects inhaled ambient air, which contained 3–6 ppb ethane. The ambient air concentration of ethane was constant during each experiment and was subtracted from the measured exhaled breath fraction.

The course of ethane concentration was analyzed under two different aspects. One aspect was to measure the profile of a single exhalation (expirogram). In this case, the recorded concentrations were plotted against the exhaled volume, which was calculated by integrating the expiratory flow (Fig. 4). For further analysis, each expirogram was divided into three parts, the first part in which the concentration is zero (phase I), the second part where the concentration rises rapidly (phase II), and the third part where the concentration increases linearly (phase III). The slope of the third part was determined by means of linear regression and normalized to the mean concentration within the third part. This value is known as the normalized alveolar slope ($S_n$).

The second aspect we focused on is the course of concentration over a time period of 30 min. Therefore, the mean concentration during phase III of each single exhalation is plotted against the time. This results in a multiexponential decay curve representing the washout process. The data is best approximated with a threefold exponential decay function. Because the subjects did not exhale any endogenous ethane, the boundary condition of an asymptote equaling zero ($y_0 = 0$) was applied. Thus the washout process can be expressed by

\[
C_{\text{alveolar}}(t) = A_1 e^{-\tau_1} + A_2 e^{-\tau_2} + A_3 e^{-\tau_3}
\]

where $C_{\text{alveolar}}$ is the mean concentration of the alveolar plateau, $A_i$ the amplitudes at time zero, $\tau_i$ the decay times, and $t$ the time. The time zero is defined by the first expiration.

Fig. 3. Representative example of an online recording of ethane, flow, CO$_2$, and O$_2$. Subjects performed predefined breathing maneuvers. Each single expiration is analyzed separately. The integrated flow gives the expired volume for the analysis of the expirograms.

Fig. 4. Recorded single exhalation expirograms for ethane and CO$_2$. 1a and 2a: expirograms for ethane at a scale of 30 and 2 ppb, respectively. 1b and 2b: corresponding expirograms for CO$_2$. In 1a, 3 phases (I to III) of expiration are marked. The additional phase IV belongs to exhaled breath beyond the functional residual volume. The gray line represents a linear regression, which is used to determine the slope of the alveolar plateau. The mean alveolar concentration is labeled with a dot.
after the washin process. The measured data was analyzed by means of a threefold exponential decay fit.

RESULTS

Characterization of the Spectrometer

The suitability of a trace gas detector for online breath analysis is mainly determined by the time resolution and sensitivity. The time resolution is given by the $T_{90}$ time. This is the time interval needed for an increase in observed concentration from 10 to 90%, which results from an instantaneous increase of the concentration from 0 to 100%. Generally, the time resolution is determined by both the exchange time of the gas setup and the averaging time (integration time) of the data processing as mentioned above. To prove the performance of CALOS, the absorbing gas was injected instantaneously at the position of the mouthpiece, which led to a jump in concentration from 0 to $3.4 \times 10^{-7}$ cm$^{-1}$. The detected course of concentration was analyzed (Fig. 5). The $T_{90}$ time determined by this measurement is $790 \pm 20$ ms. In this case, the averaging time was 300 ms so that the gas exchange time is dominating. This agrees with the theoretical exchange time of 560 ms.

The sensitivity is characterized by the minimal detectable absorption coefficient, which translates into a minimal detectable fraction of ethane of $\sim 500$ ppt for the highest time resolution (800 ms). The detection limit results from the running average processing of the measured data. It depends on the averaging time and is calculated by means of the mean quadratic standard deviation. With longer averaging times, the sensitivity will improve at the expense of a poorer time resolution. For example, if a time resolution of only 3 s is needed, the detection limit for ethane improves to $\sim 100$ ppt.

The lag time ($\Delta T$) of the CALOS analyzer is given by the time interval between the change in concentration at the position of the mouthpiece and the first detection in the absorption cell. This time was determined to be 4 s. The lag times of CO$_2$ and O$_2$ data are 1.35 and 2.2 s, respectively. These values are used to synchronize the CALOS analyzer and the capnograph. All further measurements are corrected by these lag times. The response of the breath flow measurement is instantaneous.

The specificity of the CALOS analyzer has been shown by Dahnke et al. (6). Especially with concern to CO$_2$, there are no cross interferences, because CO$_2$ has no absorption lines in the observed spectral region.

Single-Breath Analysis

All single-breath measurements showed equivalent shapes for all subjects (Fig. 4). The observed maximum concentrations covered a dynamic range from 800 ppb for the first breathing cycles to 1 ppb for the last recorded expirograms. All expirograms revealed three clearly distinguishable phases (I to III).

When subjects exhaled beyond their functional residual volume, we noticed for all subjects an additional increase in ethane concentration (phase IV) within the first 20 breathing cycles. Importantly, this additional increase in concentration was not observed in either the CO$_2$ or the O$_2$ curve.

The course of $S_n$ showed a linear increase over the first 20–30 breathing cycles and roughly a plateau for the subsequent breathing cycles (Fig. 6). Measured values for the slope of this linear increase and the mean values for the following data for $S_n$ are given in Table 1.

![Fig. 5. Demonstration of the time resolution. The response of the analyzer after an instantaneous change in the gas concentration corresponding to a jump in the absorption from zero (0%) to $3.4 \times 10^{-7}$ cm$^{-1}$ (100%) was recorded. The rise time ($\Delta T$) from 10 to 90% gives the $T_{90}$ time of 790 ms. $\Delta T$, lag time.](image)

![Fig. 6. Slope of the normalized alveolar plateau ($S_n$) for each single exhalation of the washout process. • Normalized slope obtained from the single-breath ethane expirograms (see Fig. 4); ○, normalized slope for the CO$_2$ expirograms. Lines represent linear regressions for 2 separate time domains. At the beginning of the washout process, an increase in the normalized slope for ethane is observable, whereas the $S_n$ for CO$_2$ remains fixed.](image)

Table 1. Observed progress of $S_n$

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Slope, $1^{-1}s^{-1}$</th>
<th>Plateau, liter$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>26</td>
<td>80</td>
<td>$(4.0 \pm 0.2) \times 10^{-4}$</td>
<td>$0.21 \pm 0.03$</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>100</td>
<td>$(4.3 \pm 0.3) \times 10^{-4}$</td>
<td>$0.16 \pm 0.03$</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>80</td>
<td>$(4.0 \pm 0.2) \times 10^{-4}$</td>
<td>$0.21 \pm 0.03$</td>
</tr>
</tbody>
</table>

Values are means ± SD. $S_n$, alveolar slope. *Values for the slope of $S_n$ are obtained from a linear regression weighted with the errors of the single data for $S_n$ (Fig. 6). Values for the plateau are weighted mean values for $S_n$ starting with the 25th breathing cycle.
Analysis of Washout

Figure 7 displays a representative diagram with the measured data for the ethane washout and the threefold exponential fit. The residuum is given by the difference between the data and the fit. Table 2 shows the data for each subject obtained from the fitting routine. The relatively large error for the third decay time arises from the limited sampling time of only 30 min (1,800 s) after which the ethane concentrations fell below 500 ppt. For the determination of the first amplitude and decay time, only two to four exhalations were available (breathing rate: 4 breaths/min), which leads to the uncertainty of the first amplitude. Best results are obtained for the second amplitude and decay time.

DISCUSSION

Our findings demonstrate that CALOS is a unique method for online analysis of ethane traces in human breath. Furthermore, this work is, to our knowledge, the first to demonstrate expirograms of ethane on a ppb scale. In combination with a commercially available capnograph, a simultaneous online recording of ethane concentration, breath flow, CO2, and O2 could be performed.

Single Expirograms

The expirograms for ethane, obtained from the single-breath analysis, show a shape similar to the CO2 expirograms with zero concentration at the beginning of expiration due to the physiological dead space (phase I). Then a fast increase follows, which mainly corresponds to the transition from unmixed to mixed air in the lungs (phase II). Finally a plateau with a little positive slope can be observed (phase III). This part mainly belongs to the concentration in the alveoli. Although the origin of this alveolar plateau slope has been investigated in a number of studies (13, 18), there exists no commonly accepted explanation up to now. According to Meyer et al. (13), the three most likely reasons are 1) continuing respiratory gas exchange, 2) axial partial pressure gradients in airways due to incomplete mixing and asymmetry of the respiratory airways, and 3) parallel ventilation-perfusion ratio in-homogeneity combined with sequential expiration such that regions with low ventilation-perfusion ratio preferentially empty last. The subject of inhomogeneous ventilation-perfusion distribution across the lungs was reviewed by Wagner (32).

Normalized Alveolar Plateau Slope

For the multibreath washout maneuver, our results reveal a linear increase of $S_n$, which reaches a plateau of values between 0.16 and 0.39 liter$^{-1}$. For the CO2 expirograms, we observed a $S_n$ of 0.07 liter$^{-1}$, which did not vary during the experiments.

Table 2. Decay times and amplitudes for the washout of ethane

<table>
<thead>
<tr>
<th>Subject</th>
<th>$A_1$, ppb</th>
<th>$\tau_1$, s</th>
<th>$A_2$, ppb</th>
<th>$\tau_2$, s</th>
<th>$A_3$, ppb</th>
<th>$\tau_3$, s</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>547 ± 23</td>
<td>18.5 ± 0.5</td>
<td>481 ± 5</td>
<td>53.6 ± 0.3</td>
<td>2.5 ± 0.2</td>
<td>1,200 ± 200</td>
</tr>
<tr>
<td>2</td>
<td>310 ± 210</td>
<td>11.8 ± 3.2</td>
<td>341 ± 5</td>
<td>38.6 ± 0.3</td>
<td>6.0 ± 0.5</td>
<td>370 ± 27</td>
</tr>
<tr>
<td>3</td>
<td>307 ± 6</td>
<td>23.6 ± 0.3</td>
<td>410 ± 2</td>
<td>55.1 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>1,770 ± 50</td>
</tr>
</tbody>
</table>

Values are means ± SD. $A_1$–$A_3$, amplitudes for the threefold exponential decay function; $\tau_1$–$\tau_3$, decay times for the threefold exponential decay function; ppb, parts per billion. *Values for the washout process are obtained from the threefold exponential decay fit.
The linear increase of $S_n$ during a multibreath washout is predicted by a model introduced by Tawhai and Hunter (31) for the simulation of the gas transport in the human lungs. According to this, the main reason for the change of $S_n$ within the first breathing cycles is the asymmetry of the conducting airways. Our investigations of the change of $S_n$ within the ethane washout maneuver are in qualitative agreement with these findings, especially regarding the linear increase over the first breathing cycles. The initial increase of $S_n$ can also be found in the results from Crawford et al. (5) obtained for multibreath nitrogen washout experiments. When the subjects exhaled beyond their functional residual volume, we noticed an additional increase of the ethane concentration \( \text{phase IV} \). The origin of this increase is unclear but might be related to a sequential emptying of the lungs.

**Washout After Exposure**

The washout processes of volatile organic compounds through the lungs is presumed to follow a multiexponential decay function where the different decay times are related to hypothetical compartments in the human organism, such as the blood, the vessel-rich tissue, vessel-poor tissue, and adipose tissue. Wallace et al. (33) developed a model for the course of expired concentration arising from such multiple compartments with different capacities and release times. For the analysis of the elimination process for ethane after a controlled inhalation exposure, we examined each single expirogram and determined the mean concentration of the alveolar plateau. This allows a precise determination of the expired concentration and the time of expiration. Previous studies on other volatile compounds employed sample volumes to collect the breath for later analysis (12). Although these studies made use of off-line techniques, our method is able to monitor the washout process online. Our results for the multibreath washout analysis reveal that we could recover three different compartments after an exhibition to 1 ppm ethane for 5 min and a subsequent observation period of 30 min. The least uncertainty was obtained for the second compartment (second decay time \( \sim 1 \) min), which most likely represents the blood. The third compartment (third decay time \( \sim 12 \) min) refers to highly perfused tissue, whereas the first compartment (first decay time \( \sim 20 \) s) can be attributed most likely to the washout of the lungs themselves. The decay times for the second and the third compartment presented here agree reasonably well with other studies on washout processes for volatile organic compounds (12, 21, 22). A decay time for a washout of the lungs was not reported there.

**Limitation of This Work**

We are aware of the fact that this work does provide only limited data on the physiology of ethane exhalation. First, we examined only three subjects, who performed an ethane washin and washout process. Second, a washin time of only 5 min was most likely not enough to equilibrate the subjects with ethane. Third, subjects performed a rather artificial breathing maneuver with a tidal volume of 2.5 liters to guarantee reproducibility. Nevertheless, the applied washin and washout maneuvers demonstrate the suitability of the CALOS analyzer for fast and sensitive ethane detection in exhaled breath.

**Conclusion and Perspectives**

We demonstrated a novel method for rapid and ultrasensitive analysis of ethane traces in exhaled human breath. The CALOS method enables online quantifying of ethane traces down to 500 ppt with a time resolution better than 800 ms. This unique system is capable of recording ethane expirograms of single exhalations, and it proved to be well suited for the precise investigation of the sloping alveolar plateau. Because ethane is a volatile marker for lipid peroxidation, our analyzer provides a promising noninvasive tool for online monitoring of the oxidative stress status. For example, the analyzer could be connected to a mechanical ventilator. However, this would require a modified gas system to reduce the present sample flow rate to proper values, since ventilation will seriously be affected by extraction rates on the order of 1,000 ml/min.

Because the method is based on the universal principle of infrared spectroscopy, it is not limited to ethane but may be used with slight modifications for many other constituents of exhaled breath, which are present in trace concentrations, e.g., acetone, CO, etc.

The present setup is bound to the laboratory. However, for future evaluations of the ethane exhalation as an indicator for in vivo lipid peroxidation in clinical studies, a mobile CALOS system is desirable. To provide such a mobile analyzer, a compact infrared laser must be developed that will replace the bulky CO laser in the near future. This work is currently in progress in our group (23, 30).

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**DISCLOSURES**

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